

#9118 Store at -20°C

MARK2 Antibody

✓ 100 µl
(10 Western mini-blots)

Orders ■ 877-616-CELL (2355)
orders@cellsignal.com
Support ■ 877-678-TECH (8324)
info@cellsignal.com
Web ■ www.cellsignal.com

New 03/08

This product is for *in vitro* research use only and is not intended for use in humans or animals.

Applications	Species Cross-Reactivity*	Molecular Wt.	Source
W Endogenous	H, M, R, (Mk)	78, 82 kDa	Rabbit

Background: Microtubule associated proteins regulate the stability of microtubules and control processes such as cell polarity/differentiation, neurite outgrowth, cell division and organelle trafficking (1). The MARK (MAP/microtubule affinity-regulating kinases) family (MARK1-4) of serine/threonine kinases was identified based on their ability to phosphorylate microtubule-associated proteins (MAPs) including tau, MAP2 and MAP4 (2-6). MARK proteins phosphorylate MAPs within their microtubule binding domains, causing dissociation of MAPs from microtubules and increased microtubule dynamics (2-4). In the case of tau, phosphorylation has been hypothesized to contribute to the formation of neurofibrillary tangles observed in Alzheimer disease. Overexpression of MARK leads to hyperphosphorylation of MAPs, morphological changes and cell death (4). The tumor suppressor kinase LKB1 phosphorylates MARK and the closely related AMP-kinases within their T-loops, leading to increased activity (7).

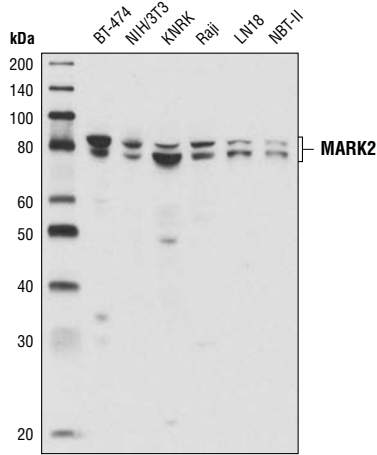
MARK2 (4), also termed as Par-1 (8) and EMK1 (9), contributes to cellular polarity, cell cycle progression, microtubule dynamics, and neurite outgrowth. The MARK2 gene encodes at least two alternatively spliced isoforms that are co-expressed in various cell lines (10). Substrates of MARK2 include microtubule associated protein (MAPs), tau, histone deacetylases (11), and Rab11-FIP2 (12). Knockout studies suggest that MARK2 plays an essential role in immune system function (13), glucose homeostasis (14), and learning and memory (15).

Specificity/Sensitivity: MARK2 Antibody detects endogenous levels of total MARK2 protein. No cross reactivity is observed with other MARK family members.

Source/Purification: Polyclonal antibodies are produced by immunizing rabbits with a synthetic peptide (KLH-coupled) corresponding to residues surrounding Lys430 of human MARK2. Antibodies were purified by protein A and peptide affinity chromatography.

Background References:

- (1) Drubin, D.G. and Nelson, W.J. (1996) *Cell* 84, 335-344.
- (2) Illenberger, S. et al. (1996) *J. Biol. Chem.* 271, 10834-10843.
- (3) Drewes, G. et al. (1995) *J. Biol. Chem.* 270, 7679-7688.



Western blot analysis of extracts from various cell lines using MARK2 Antibody.

- (4) Drewes, G. et al. (1997) *Cell* 89, 297-308.
- (5) Kato, T. et al. (2001) *Neoplasia* 3, 4-9.
- (6) Trinczek, B. et al. (2004) *J. Biol. Chem.* 279, 5915-5923.
- (7) Lizcano, J. M. et al. (2004) *EMBO J.* 23, 833-843.
- (8) Guo, S. and Kempfues, K.J. (1995) *Cell* 81, 611-620.
- (9) Inglis, J.D. et al. (1993) *Mamm. Genome* 4, 401-403.
- (10) Espinosa, L. and Navarro, E. (1998) *Cytogenet. Cell Genet.* 81, 278-282.
- (11) Dequiedt, F. et al. (2006) *Mol. Cell. Biol.* 26, 7086-7102.
- (12) Ducharme, N.A. et al. (2006) *Mol. Biol. Cell* 17, 3625-3637.
- (13) Hurov, J.B. et al. (2001) *Mol. Cell. Biol.* 21, 3206-3219.
- (14) Hurov, J.B. et al. (2007) *Proc. Natl. Acad. Sci. USA* 104, 5680-5685.
- (15) Segu, L. et al. (2008) *Neurobiol. Aging* 29, 231-240.

Entrez-Gene ID #2011
Swiss-Prot Acc. #Q7KZ17

Storage: Supplied in 10 mM sodium HEPES (pH 7.5), 150 mM NaCl, 100 µg/ml BSA and 50% glycerol. Store at -20°C. Do not aliquot the antibody.

*Species cross-reactivity is determined by Western blot.

Recommended Antibody Dilutions:

Western blotting 1:1000

Companion Products:

- Phospho-MARK Family (Activation Loop) Antibody #4836
- MARK4 Antibody #4834
- MARK1 Kinase #7352
- MARK3 Kinase #7354
- Phototope®-HRP Western Blot Detection System, Anti-rabbit IgG, HRP-linked Antibody #7071
- Anti-rabbit IgG, HRP-linked Antibody #7074
- Prestained Protein Marker, Broad Range (Premixed Format) #7720
- Biotinylated Protein Ladder Detection Pack #7727
- 20X LumiGLO® Reagent and 20X Peroxide #7003

IMPORTANT: For Western blots, incubate membrane with diluted antibody in 5% w/v BSA, 1X TBS, 0.1% Tween-20 at 4°C with gentle shaking, overnight.

Western Immunoblotting Protocol (Primary Antibody Incubation in BSA)

For Western blots, incubate membrane with diluted antibody in 5% w/v BSA, 1X TBS, 0.1% Tween-20 at 4°C with gentle shaking, overnight.

A Solutions and Reagents

NOTE: Prepare solutions with Milli-Q or equivalently purified water.

- 1X Phosphate Buffered Saline (PBS)
- 1X SDS Sample Buffer:** 62.5 mM Tris-HCl (pH 6.8 at 25°C), 2% w/v SDS, 10% glycerol, 50 mM DTT, 0.01% w/v bromophenol blue or phenol red
- Transfer Buffer:** 25 mM Tris base, 0.2 M glycine, 20% methanol (pH 8.5)
- 10X Tris Buffered Saline (TBS):** To prepare 1 liter of 10X TBS: 24.2 g Tris base, 80 g NaCl; adjust pH to 7.6 with HCl (use at 1X).
- Nonfat Dry Milk (weight to volume [w/v])
- Blocking Buffer:** 1X TBS, 0.1% Tween-20 with 5% w/v nonfat dry milk; for 150 ml, add 15 ml 10X TBS to 135 ml water, mix. Add 7.5 g nonfat dry milk and mix well. While stirring, add 0.15 ml Tween-20 (100%).
- Wash Buffer:** 1X TBS, 0.1% Tween-20 (TBS/T)
- Bovine Serum Albumin (BSA)
- Primary Antibody Dilution Buffer:** 1X TBS, 0.1% Tween-20 with 5% BSA; for 20 ml, add 2 ml 10X TBS to 18 ml water, mix. Add 1.0 g BSA and mix well. While stirring, add 20 µl Tween-20 (100%).
- Phototope[®]-HRP Western Blot Detection System #7071:** Includes biotinylated protein ladder, secondary anti-rabbit (#7074) antibody conjugated to horseradish peroxidase (HRP), anti-biotin antibody conjugated to HRP, LumiGLO[®] chemiluminescent reagent and peroxide.
- Prestained Protein Marker, Broad Range (Premixed Format) #7720
- Biotinylated Protein Ladder Detection Pack #7727
- Blotting Membrane:** This protocol has been optimized for nitrocellulose membranes, which CST recommends. PVDF membranes may also be used.

B Protein Blotting

A general protocol for sample preparation is described below.

- Treat cells by adding fresh media containing regulator for desired time.
- Aspirate media from cultures; wash cells with 1X PBS; aspirate.
- Lyse cells by adding 1X SDS sample buffer (100 µl per well of 6-well plate or 500 µl per plate of 10 cm diameter plate). Immediately scrape the cells off the plate and transfer the extract to a microcentrifuge tube. Keep on ice.
- Sonicate for 10–15 seconds to shear DNA and reduce sample viscosity.
- Heat a 20 µl sample to 95–100°C for 5 minutes; cool on ice.
- Microcentrifuge for 5 minutes.
- Load 20 µl onto SDS-PAGE gel (10 cm x 10 cm).

NOTE: CST recommends loading prestained molecular weight markers (#7720, 10 µl/lane) to verify electrotransfer and biotinylated protein ladder (#7727, 10 µl/lane) to determine molecular weights.

- Electrotransfer to nitrocellulose or PVDF membrane.

C Membrane Blocking and Antibody Incubations

NOTE: Volumes are for 10 cm x 10 cm (100 cm²) of membrane; for different sized membranes, adjust volumes accordingly.

- (Optional) After transfer, wash nitrocellulose membrane with 25 ml TBS for 5 minutes at room temperature.
- Incubate membrane in 25 ml of blocking buffer for 1 hour at room temperature.
- Wash three times for 5 minutes each with 15 ml of TBS/T.
- Incubate membrane and primary antibody (at the appropriate dilution) in 10 ml primary antibody dilution buffer with gentle agitation overnight at 4°C.
- Wash three times for 5 minutes each with 15 ml of TBS/T.
- Incubate membrane with HRP-conjugated secondary antibody (1:2000) and HRP-conjugated anti-biotin antibody (1:1000) to detect biotinylated protein markers in 10 ml of blocking buffer with gentle agitation for 1 hour at room temperature.
- Wash three times for 5 minutes each with 15 ml of TBS/T.

D Detection of Proteins

- Incubate membrane with 10 ml LumiGLO[®] (0.5 ml 20X LumiGLO[®], 0.5 ml 20X Peroxide and 9.0 ml Milli-Q water) with gentle agitation for 1 minute at room temperature.

NOTE: LumiGLO[®] substrate can be further diluted if signal response is too fast.

- Drain membrane of excess developing solution (do not let dry), wrap in plastic wrap and expose to x-ray film. An initial 10-second exposure should indicate the proper exposure time.

NOTE: Due to the kinetics of the detection reaction, signal is most intense immediately following LumiGLO[®] incubation and declines over the following 2 hours.